Adipose tissues from various anatomical sites are characterized by different patterns of gene expression and regulation

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We have shown previously the presence of brown adipocytes among white fat pads, and proposed the existence of a spectrum of adipose depots according to the abundance of brown fat cells [Cousin, Cinti, Morroni, Raimbault, Ricquier, Pénicaud and Casteilla (1992) J. Cell Sci. **103**, 931–942]. In this study, we tried to characterize this spectrum better. We determined in several adipose depots (i) the richness of pre-adipose cells, as assessed by A2COL6 mRNA levels; (ii) whether a fat pad was characterized by a pattern of mRNA expression; (iii) whether this pattern was close related to abundance of brown adipocytes, and (iv) whether the regulation of this pattern by catecholamines under cold exposure or β-agonist treatment was similar in the different pads. This was achieved by studying proteins involved in glucose and lipid metabolism such as insulin-sensitive glucose transporter (GLUT4), fatty acid synthase, lipoprotein lipase and fatty acid binding protein aP2, as well as β_3-adrenergic-receptor expression. Among white adipose depots, the periovarian fat pad was characterized by the highest content of pre-adipocytes and of brown adipocytes, and inguinal fat by the highest lipogenic activity potential. There was no close correlation between β_3-adrenergic-receptor expression and brown adipocyte content in the tissues, as measured by the degree of uncoupling protein (UCP) gene expression. However, in pads expressing UCP mRNA, mRNA levels of β_3-adrenergic receptor and other markers were increased in parallel. Under cold exposure or β_3-agonist treatment, a specific up-regulation of GLUT4 expression was observed in interscapular brown adipose tissue. The regional difference described in this study, could participate in preferential fat-pad growth under physiological conditions as well as in pathological situations.

INTRODUCTION

Brown adipose tissue (BAT) functions as a heat-producing organ [1], whereas white adipose tissue (WAT) constitutes the primary energy-storage compartment. Lipogenic and lipolytic pathways are common features of these two types of adipose tissues, in which glucose and lipid metabolism are, in part, controlled by the sympathetic nervous system, via a β-adrenergic pathway [2–5].

Proliferation from pre-adipocytes, in BAT, occurs under cold stimulation or catecholamine treatment, whereas WAT development is observed after high-fat-diet administration, or during pathological situations like obesity. Brown adipocyte terminal differentiation leads to expression of the uncoupling protein (UCP), which is specific to mitochondria in brown fat cells and responsible for the thermogenic role of BAT [6]. White adipocyte differentiation leads to the appearance of enzymes required for triglyceride accumulation, although no enzymic marker specific to white adipocytes is yet known.

For practical convenience, most studies on adipose tissues have been investigated on a single depot: epididymal or subcutaneous for WAT, interscapular BAT (IBAT) for BAT. Nevertheless, some results indicate that differences in metabolic activity exist depending on the localization of the tissue. Thus, in adult rodents, subcutaneous depots have the highest resting rate of lipolysis and the lowest response to both noradrenaline and insulin [7,8]. Furthermore it has been shown, in man, that abdominal and subcutaneous tissues respond differently to steroids [9]. Moreover, many results indicated that cellular populations, as well as specific activities of adipocytes, were not similar between the different white fat pads. Thus, studies *in vivo* suggest that the pattern of protein expression in mature cells could vary according to the anatomical location of fat pads: for example the α_2-adrenergic receptor was expressed more in subcutaneous than in intra-abdominal deposits [10], in contrast with the insulin receptor [11]. We have recently shown, in rats, the presence of variable amounts of brown adipocytes in fat pads considered to be WAT, and a spectrum of adipose tissues has been described, from IBAT to inguinal WAT [12]. Previous studies *in vitro* have shown that adipocyte precursors from different sites revealed heterogeneity in their capacities for replication and proliferation [13–15]. The amount of pre-adipose cells, measured by A2COL6 expression, varied between inguinal, parametrial and perirenal fat pads [16]. Thus, these results on the developmental potential could be related to abundance of pre-adipocytes in different adipose depots. Altogether, these results suggest that differences in adipocyte precursor pools, and in the pattern of gene expression and regulation, contribute to the dissimilar properties of the fat pads, and could explain the differential growth of adipose depots in physiological and pathological situations such as obesity.

The aims of this study were to determine, in several adipose depots, (i) the abundance of pre-adipose cells; (ii) whether a fat pad is characterized by a pattern of expression of mRNA encoding for proteins involved in metabolism; (iii) whether this pattern is closely related to abundance of brown adipocytes; and (iv) whether the regulation of this pattern by catecholamines is similar in the different pads. The study of mRNAs by a Northern-blot experiment allowed us to determine quickly and simultaneously a pattern of gene expression.

Abbreviations used: BAT, brown adipose tissue; WAT, white adipose tissue; UCP, uncoupling protein; IBAT, interscapular BAT; GLUT4, insulin-sensitive glucose transporter; FAS, fatty acid synthase; LPL, lipoprotein lipase; aP2, fatty acid binding protein aP2.

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MATERIALS AND METHODS

Animals

Female Wistar rats (8 weeks old) were housed in animal quarters in which the temperature was maintained at 24 °C with free access to food (65 % carbohydrate, 11 % fat, 24 % protein, w/w; UAR, Villemoisson, France) and water, and with the light on from 07:00 to 19:00. One experimental group was kept at a constant cold temperature (4 °C) for 24 h and rats were caged individually. Another group was treated three times daily, with a β_3-adrenergic-receptor agonist (D7114, ICI Pharmaceuticals, Alderley Park, Cheshire, U.K., 10 mg/kg, intraperitoneally), and animals were killed 24 h after the first injection. Animals were killed by cervical dislocation, and the different fat pads were rapidly removed in their entirety and immediately frozen in liquid N_2.

Northern-blot analysis

Adipose tissues were reduced to powder in liquid N_2 and an aliquot (0.3–1 g) was used to extract total RNA using guanidine thiocyanate [17]. RNA concentrations were determined by measuring absorbance at 260 nm, and the RNAs were stored in water containing diethylpyrocarbonate (0.02 %) at −80 °C until use. Northern-blot experiments were performed according to Sambrook et al. [18], with 20 μg of total RNA from IBAT, periovarian retroperitoneal, and inguinal fat pads. The same plots were hybridized with several probes. The probes were labelled with ^{32}P using a Megaprime-labelling-system kit (Amersham International, Amersham, Bucks., U.K.). They were either raised in our groups, β_3-adrenergic receptor [19] and A2COL6 [20], or kindly provided by Dr. D. James for insulin-sensitive glucose transporter (GLUT4) [21], Dr. A. G. Goodridge for fatty acid synthase (FAS) [22], Dr. Schotz for lipoprotein lipase (LPL) [23], and Dr. Spiegelman for fatty acid binding protein (aP2) [24]. To verify the amount of total RNA in each lane, blots were hybridized with a synthetic oligonucleotide specific for ribosomal 18S RNA [25]. The blots were then exposed for between 4 h and 4 days at −80 °C with intensifying screens. Quantification was performed by scanning densitometry (Hoefer Scientific Instruments, San Francisco, CA, U.S.A.). Efficiency of cold exposure and β_3-agonist treatment was characterized by UCP expression [26].

Analytical techniques

Blood samples were centrifuged and glucose and plasma insulin concentrations were measured by means of a glucose analyser (Beckman 2, Palo Alto, CA, U.S.A.) or by a r.i.a. kit (SB-INSI-5, ORIS, Gif/Yvette, France) using rat insulin as the standard. Proteins were quantified using the Bio-Rad protein assay (Bio-Rad, Munich, Germany).

Statistical analysis

Results were expressed as means ± S.E.M. Statistical analysis was performed using Student's t test for unpaired data.

RESULTS AND DISCUSSION

Pattern of gene expression in 8-day-old female rats

A2COL6 is the α_2-chain of collagen subtype VI, and is transiently expressed in pre-adipocytes during adipose conversion; its level of mRNA expression was used to estimate the pre-adipocytes content of each fat pad [20]. As shown in Table 1, when expressed as a percentage of IBAT values, the expression of A2COL6 was higher in periovarian pads than in IBAT, retroperitoneal inguinal depots. This suggests a greater pre-adipocyte content and proliferation potential in periovarian depots. We have previously shown that under cold exposure, UCP expression and the amount of differentiated brown fat cells increased in periovarian depot [12]. Thus, this increase could be explained by a recruitment of the important amount of pre-adipocytes present in this fat pad. This pattern of expression of adipocyte precursor cells is quite different to that reported in mice [16] in which a higher level of expression was found in inguinal pads than in parametrial and perirenal depots. This difference between rats and mice could be explained by a difference in tissue phenotypes, thus, in mice, the expression of UCP increased significantly in the inguinal fat pad during cold exposure [27].

Under standard conditions, mature adipocytes are characterized by the expression of proteins involved in glucose and lipid metabolism. In order to establish, for different fat pads, a characteristic pattern of gene expression, GLUT4, FAS, LPL and aP2 mRNA levels were assessed as indices of glucose and lipid metabolism respectively. The level of β_3-adrenergic-receptor mRNA was used as an index of catecholamine sensitivity since it is the predominant adrenergic receptor of BAT and WAT [19]. The level of expression of each marker was studied in the different fat pads, and expressed as a percentage of BAT values (Table 1). Fat pads could be classified on the basis of mRNA-transcript abundance. For all markers studied, the most important levels of mRNA were found in IBAT. It was noteworthy that the LPL mRNA concentration was not statistically different in the various white fat pads, when the levels of the other marker mRNA was higher in periovarian, than in retroperitoneal and inguinal depots. The aP2 mRNA level was similar in periovarian fat pads and IBAT.

The results also showed that the level of β_3-adrenergic-receptor mRNA was lower in all WAT depots than in IBAT, as well as the UCP mRNA level, as previously described [12]. In pads which expressed UCP mRNA, β_3-receptor and other marker mRNA levels increased in parallel. However, there was no close correlation between β_3-adrenergic-receptor and UCP mRNA values. This is reminiscent of results obtained in adipose cell line culture, in which expression of β_3-adrenergic receptors was found without UCP expression [28,29]. The differences between IBAT and other fat pads could not be explained by their different contents of brown fat cells, revealed by UCP expression. These results suggest that, in rodents, the β_3-adrenergic-receptor gene was expressed not only in brown but also in white adipocytes, although at a lower level [19,28].

Table 1 Relative values of marker mRNA levels in different fat pads, expressed as percentages of IBAT

Results are expressed as means ± S.E.M. (n = 4 to 6 rats). *$P < 0.05$ in comparison to values observed in IBAT. Abbreviations: β_3-AR: β_3-adrenergic receptor; PO, periovarian; RP, retroperitoneal; Ing, inguinal; U, undetectable.

	BAT	PO	RP	Ing
A2COL6	100 ± 10	160 ± 21*	64 ± 11*	49 ± 5*
β_3-AR	100 ± 15	31 ± 7*	21 ± 6*	U
GLUT4	100 ± 54	53 ± 15	48 ± 13	5 ± 1*
LPL	100 ± 22	46 ± 19	63 ± 28	40 ± 6*
FAS	100 ± 63	53 ± 15	48 ± 10	9 ± 2*
aP2	100 ± 2	92 ± 3	58 ± 13*	4 ± 0.3*
UCP	100	10	U	U

The expression of the results as a percentage of the aP2 values allowed comparison of different fat pads for all the markers, since aP2 was specific to mature adipocytes, and a late marker of terminal adipocyte differentiation [24]. In this case, the inguinal profile was different from that of other depots, due to relatively high expression of LPL and FAS. These differences could be due to changes in either the specific activities of mature adipocytes, or the differentiation state of adipose cells.

Taken together, these results demonstrate regional differences in the gene expression of fat pads in 8-week-old control rats which could be explained, in part, by the different specific activities of adipocytes. Anatomical characteristics (innervation, vascularization), could emphasize these regional disparities, as well as quantitative or qualitative differences in response to stimulatory factors.

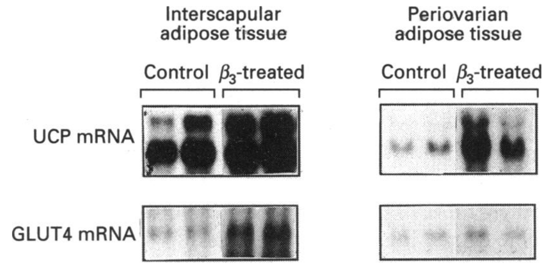

Figure 1 Northern-blot analysis of UCP and GLUT4 mRNA levels in intercapular and periovarian fat pads of rats.

Rats were injected three times during 24 h either with physiological serum (control rats) or with D7114 (10 mg/kg) (β_3-agonist-treated).

Regulation

Acute exposure of rats to cold induces, in BAT, a stimulation of metabolic activity and an increase in noradrenaline content and turnover [30,31]. Catecholamines are known to modulate glucose and lipid metabolism in white and brown adipocytes. Therefore, the possible influence of cold exposure and catecholamines on the pattern of mRNA expression was investigated.

In IBAT, as well as in all WAT depots, no significant variations in transcript mRNA levels were found for all the markers studied, except for UCP, and GLUT4, in IBAT. UCP, FAS and GLUT4 mRNA levels after cold exposure are described in Table 2. Results on FAS and LPL mRNA (not shown) were quite surprising. In a similar situation, an increase in LPL activity in BAT [32,33], and a stimulation of fatty acid synthesis in BAT and in WAT have been described when animals were maintained at 4 °C for 24 h or 3 weeks respectively [34]. The recruitment of precursors or a post-transcriptional regulation, following cold adaptation could explain these differences.

GLUT4 mRNA expression was markedly enhanced after cold exposure in IBAT, while no change was observed in WAT whatever the location of the fat pad. The great increase in the level of GLUT4 mRNA in IBAT was in agreement with results obtained by Nikami et al. [35]. The discrepancy observed in the degree of stimulation of GLUT4 mRNA levels by cold (ten in the present study versus two) could be explained by the length of cold exposure (24 h versus 10 days at 4 °C). This demonstrates a tissue-specific regulation of the GLUT4 gene in IBAT. These results on GLUT4 mRNA expression in IBAT and WAT were in agreement with previous studies reporting either an increase or no change, in glucose utilization after 4–48 h of cold exposure [6,36,37]. However, our results are in contrast with those that have been described for insulin regulation of GLUT4 expression. It is well known that GLUT4 expression is controlled by insulin in WAT and skeletal muscles [38]. In cold-exposed animals, the plasma insulin level was decreased (12 ± 2 versus 24 ± 2 μ-unit/ml, $P < 0.01$; [39]) while an increased GLUT4 mRNA level was described. These results are consistent with an increase in glucose utilization or GLUT4 expression upon cold exposure [6,35,36,40], and indicate that the stimulatory effect of cold exposure on glucose utilization or GLUT4 expression in BAT is probably not mediated by circulating insulin, although an additional regulation, probably by catecholamines, occurred.

Table 2 UCP, GLUT4 and FAS mRNA concentrations in IBAT, periovarian (PO) and inguinal (Ing) white fat pads of female rats exposed to cold (4 °C) for 24 h compared with rats maintained at 24 °C

Results are expressed as means ± S.E.M. ($n = 4$ to 6 rats). $^*P < 0.05$ in comparison with values observed at 24 °C. Abbreviation: U, undetectable.

Marker	Treatment temperature (°C)	IBAT	PO	Ing
UCP	24	1 ± 0.4	1 ± 0.3	U
	4	$3.4 \pm 0.7^*$	$4.6 \pm 1.2^*$	U
GLUT4	24	1 ± 0.5	1 ± 0.2	1 ± 0.1
	4	$10.9 \pm 0.8^*$	1 ± 0.2	0.8 ± 0.1
FAS	24	1 ± 0.6	1 ± 0.3	1 ± 0.2
	4	1.4 ± 0.7	0.7 ± 1.2	0.5 ± 0.2

To investigate if the cold-exposure effect on GLUT4 expression was mediated by the β-adrenergic response and particularly via the β_3-adrenergic pathway, animals were treated with a β_3-agonist for 24 h (Figure 1). Plasma insulin levels were not changed in treated rats (21 ± 1 μ-unit/ml) in comparison with controls (24 ± 2 μ-unit/ml). UCP mRNA expression was used as the control, and was increased by treatment in IBAT and in the periovarian fat pad. GLUT4 mRNA levels were slightly increased in IBAT, after 24 h of β_3-agonist treatment. This was in accordance with results obtained with Ro 16-8714 on lean Zucker rats [41]. However, whereas cold exposure and β_3-agonist treatment led to the same stimulation of UCP expression, the former induced a more effective stimulation of GLUT4 expression than the latter (stimulation by a factor of ten as opposed to two). This suggests that in IBAT, β_3-adrenergic-receptor stimulation was not the only pathway for GLUT4 regulation under cold exposure.

In WAT, GLUT4 mRNA levels were not affected by treatment with a β_3-agonist for 24 h. An increase of glucose transport has been shown previously, but no variation in the amount of GLUT4 was observed [3,4]. These results demonstrate that cold exposure, as well as β_3-agonist treatment, had no effect on GLUT4 expression in WAT, and confirm the tissue-specific regulation of the GLUT4 gene in fat pads [42]. It is noteworthy that GLUT4 gene regulation by β_3 agonist is different according to the adipose depots analysed, whereas the β_3-receptor gene is the major β-adrenergic receptor expressed in rodent fat pads.

These results, and those in our previous work [12], indicate that a particular pattern of cells and gene expression exists in every adipose tissue. Tissue-specific regulation of GLUT4 expression by catecholamines could be another factor characterizing IBAT versus other adipose depots.

These regional disparities in gene expression of various fat pads suggest the existence of differences in their metabolic activities. Thus, the inter-depot differences in GLUT4, FAS and

LPL expression could indicate that in terms of fat accumulation intra-abdominal tissues depend more on lipogenesis, whereas subcutaneous tissue relies more upon capture of free fatty acids. This could also be important in terms of pathology and treatment. Thus, it could be related to the preferential growth of subcutaneous adipose depots in obese rats [43]. Furthermore, the differential expression of the β_3-adrenergic receptor among the depots made them more or less susceptible to a potential beneficial effect of specific drugs. In summary these results stress both the fact that adipose depots are not similar and the importance of characterizing the different adipose depots in order to have a better understanding of their specific development and metabolism.

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